Role of *kdr* and Esterase-Mediated Metabolism in Pyrethroid-Resistant Populations of *Haematobia irritans irritans* (Diptera: Muscidae) in Brazil

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ABSTRACT The horn fly, *Haematobia irritans irritans* (L.) (Diptera: Muscidae), has become a problem for Brazilian cattle producers even though its introduction into Brazil is relatively recent. Failure to control this cattle pest is becoming a concern, and horn fly populations from several ranches from the state of Mato Grosso do Sul were surveyed for pyrethroid resistance. Susceptibility bioassays revealed that cypermethrin resistance was widespread and reached high levels in horn fly populations throughout the state, with resistance factors (RFs) ranging from 50.4 to 704.8. Synergist bioassays failed to detect a major role for esterases as a pyrethroid resistance mechanism in these populations, except for the highly pyrethroid-resistant Estrela do Oeste population (RF = 704.8). The *kdr* sodium channel gene mutation was not detected in eight of the 13 populations, but <7% of individuals from four populations and 50% of the flies from Estrela do Oeste exhibited this mutation. Neither the *superkdr* sodium channel gene mutation nor a resistance-associated gene mutation in the *HiαE7* carboxylesterase were found in any of the fly populations. Although target site insensitivity (*kdr*) and esterase-mediated metabolism occur in horn fly populations from Mato Grosso do Sul state, it seems that they are not the major mechanism causing pyrethroid resistance in most of these populations.

KEY WORDS *Haematobia irritans irritans*, pyrethroid resistance, *kdr*, insecticide bioassays

The horn fly, *Hematobia irritans irritans* (L.) (Diptera: Muscidae), is considered to be a major insect pest of cattle in many parts of the world. Economic losses to the Brazilian cattle industry of US$150 million have been attributed to this fly (Grisi et al. 2002), and the primary means of horn fly control has been based upon the use of insecticides. However, the development of resistance and control failures resulting from resistance have become common in many parts of the horn fly's distribution range (Kunz and Schmidt 1985, Kunz et al. 1995, Barros 2004).

There are several possible mechanisms whereby arthropods can develop pesticide resistance, and there is evidence that several of these mechanisms can be important to resistant populations of the horn fly (Sparks et al. 1985). Xu and Bull (1995) reported esterase-mediated pyrethroid metabolism was elevated in pyrethroid-resistant laboratory strains of horn flies. Esterase activity was surveyed in several populations of pyrethroid-susceptible and -resistant horn flies from several locations in the United States, and a complex pattern of esterase expression was speculated to contribute to pyrethroid resistance (Guerrero et al. 1999, Pruett et al. 2000, Pruett et al. 2001). Sheppard (1995) found that piperonyl butoxide (PBO)-suppressible pesticide resistance was a major mechanism in cyhalothrin-selected horn flies and attributed this to an oxidative metabolic enzyme system that inactivates the pyrethroid. Jamroz et al. (1998) correlated the presence of the *kdr* and *superkdr* gene mutations in the sodium channel, the target site of pyrethroids, with pyrethroid resistance in both field and laboratory populations of horn flies.

The horn fly was a relatively recent introduction to Brazil (Valério and Guimarães 1983), and problems with insecticide resistance in that region are becoming increasingly common in Brazil and neighboring countries. For example, pyrethroid resistance in Argentina was reported within 4 yr of the introduction of that class of chemicals to control the horn fly (Sheppard and Torres 1998), and the incidence of resistance in that country has been rising (Guglielmone et al. 2001). The objective of our research was to use synergist bioassays and a multiplex polymerase chain reaction (PCR) assay to determine the occurrence, levels, and role of target site insensitivity and esterase-mediated metabolism as mechanisms of horn fly pyrethroid resistance in the Brazilian state of Mato Grosso do Sul.

Materials and Methods

Horn Flies. Adult horn flies were collected at various times from several locations in Mato Grosso do Sul

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Table 1. Sampling locations and pyrethroid bioassay results from horn fly populations in central Brazil

Location[a]	Date	Bioassay treatment	$LC_{50-2\ h}$ (95% FL)[b] $\mu g/cm^2$	Slope (SE)[c]	RF[d]
Initial surveys for permethrin resistance					
Ciaco, Corumbá, MS	10/11/2000	Permethrin	4.90 (4.06–5.45)[e]	8.25 (1.77)	1.9
Nhumirim, Corumbá, MS	10/25/2000	Permethrin	3.98 (3.09–4.92)[e]	5.20 (0.53)	1.5
Monjolo, Cuiabá, MT	11/9/2000	Permethrin	4.90 (3.21–7.14)[e]	4.40 (0.35)	1.9
Cristo, Miranda, MS	5/16/2001	Permethrin	2.05 (1.64–2.58)	4.11 (0.35)	0.8
Kerrville Susceptible Reference		Permethrin	2.59 (2.42–2.79)	6.30 (0.57)	—
Surveys for cypermethrin resistance					
Santa Cruz, Aquidauana, MS	2/19/2002	Cypermethrin	21.69 (14.66–30.93)[e]	2.84 (0.20)	50.4
Taboco, Aquidauana, MS	5/8/2002	Cypermethrin	38.17 (32.85–43.93)[e]	2.49 (0.22)	88.8
Estância Sta Izabel, Porto Murtinho, MS	6/6/2002	Cypermethrin	23.74 (20.12–27.51)[e]	2.84 (0.29)	55.2
Kerrville Susceptible Reference		Cypermethrin	0.43 (0.29–0.70)	4.57 (0.37)	—
Synergist bioassays for esterase component to cypermethrin resistance					
Nhumirim, Corumbá, MS	3/24/2004	Cypermethrin	15.65 (10.22–21.89)[e]	3.80 (0.36)	78.3
		Cypermethrin + TPP	20.34 (16.41–25.35)[e]	2.40 (0.16)	135.6
Iguaçu, Aquidauana, MS	4/6/2004	Cypermethrin	39.91 (18.39–57.02)[e]	2.68 (0.37)	199.6
		Cypermethrin + TPP	33.41 (15.26–54.72)[e]	2.65 (0.19)	222.7
Bosque Belo, Aquidauana, MS	4/8/2004	Cypermethrin	23.30 (17.33–29.57)[e]	3.34 (0.37)	116.5
		Cypermethrin + TPP	28.83 (10.96–51.68)[e]	2.07 (0.19)	192.2
Santa Izabel, Aquidauana, MS	4/7/2004	Cypermethrin	23.46 (16.25–32.66)[e]	2.43 (0.19)	117.3
		Cypermethrin + TPP	33.23 (26.03–40.01)[e]	2.58 (0.36)	221.5
Estrela do Oeste, Jardim, MS	6/17/2004	Cypermethrin	140.96 (110.25–178.59)[e]	2.02 (0.16)	704.8
		Cypermethrin + TPP	57.64 (50.13–66.20)[e,f]	2.28 (0.18)	384.3
Barra Bonita, Jardim, MS	6/18/2004	Cypermethrin	81.88 (52.49–130.72)[e]	2.13 (0.13)	409.4
		Cypermethrin + TPP	53.71 (24.56–114.17)[e]	1.41 (0.10)	358.1
Kerrville Susceptible Reference		Cypermethrin	0.20 (0.11–0.28)	4.59 (0.34)	—
		Cypermethrin + TPP	0.15 (0.14–0.17)	5.64 (0.55)	—

[a] County of ranch location and state (MS, Mato Grosso do Sul state; MT, Mato Grosso state).
[b] Two-hour LC_{50}, including 95% fiducial limits (FL), determined from dose–mortality regression probit analysis as micrograms of active ingredient per filter paper area in square centimeters.
[c] Slope of dose–mortality regression line, including standard error.
[d] RF = test LC_{50} ÷ Kerrville reference susceptible colony LC_{50} used as control.
[e] Statistically significant difference compared with the Kerrville reference strain $LC_{50-2\ h}$.
[f] Statistically significant difference between synergized and nonsynergized bioassay $LC_{50-2\ h}$.

(Table 1). The Monjolo sample was collected from the neighboring state of Mato Grosso. Study sites were randomly selected depending on conditions of access to the ranches and willingness of the owners.

Susceptibility of horn fly populations was assessed by using impregnated filter papers (Sheppard and Hinkle 1987) produced at the Laboratory of Entomology of the Embrapa Pantanal by using technical permethrin (>97% [AI], FMC do Brasil, Campinas, São Paulo, Brazil and Chem Service, West Chester, PA), cypermethrin (>89% [AI], donated by Minerthal, São Paulo, Brazil), and triphenyl phosphate (90% [AI], Aldrich, Milwaukee, WI) diluted in acetone. Each insecticide kit contained three replicates of eight to 10 concentrations of permethrin (0.4–51.2 $\mu g/cm^2$) and cypermethrin or cypermethrin + triphenyl phosphate (TPP) (1.6–819.2 $\mu g/cm^2$). Bioassays with the insecticide susceptible Kerrville reference colony (maintained in vitro at the USDA Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, TX) used filter papers impregnated with cypermethrin (or cypermethrin + TPP) at 0.05–1.6 $\mu g/cm^2$. TPP was used at 5% (wt:vol) in all synergist bioassays. This synergist was used to determine metabolic esterase contributions to pyrethroid resistance. Concentration was based on previous bioassays performed on the Kerrville colony with TPP-treated papers (F.G., unpublished data) based on considerations from Scott (1990). Control papers were treated with acetone only.

Bioassays were performed according to Barros et al. (2002). Fly mortality was assessed immediately after dishes were loaded to check for early mortality due to fly collection and manipulation and also after a 2-h exposure to the insecticide. Flies for genomic assays were transferred alive to ethanol-filled vials after checking for early mortality.

Flies unable to walk were considered dead, and mortality data analyzed by POLO-PC (LeOra Software 1987). Mortalities in controls were <5% except for Iguaçu (7.2%) and Ciaco (12.7%). Resistance factors (RFs) were calculated by dividing lethal concentration (LC_{50}) from field populations by the LC_{50} from the Kerrville colony. RFs for synergized insecticide bioassays were calculated based on the LC_{50} obtained from exposure of the Kerrville colony to a similar kit including synergist. Differences in LC_{50} were considered significant when their 95% fiducial limits did not overlap.

In addition, a standard questionnaire regarding ectoparasite control was completed at each ranch where bioassays were conducted to obtain a history of insecticide use and livestock pest control practices.

Multiplex PCR Assay. A PCR-based multiplex resistance assay was used that screens individuals for target site-mediated resistance resulting from the *kdr*

Table 2. Sequences of PCR primers used to genotype individual horn flies

Primer ID	Primer sequence	Description of primer function
FG-130	TACTGTTGTCATCGGCAATC	Sus upstream *kdr* diagnostic
FG-134	TACTGTTGTCATCGGCAATT	Res upstream *kdr* diagnostic
FG-138	CAATATTACGTTTCACCCAG	Sus/Res downstream *kdr* primer
FG-235	CTTCGTGTATTCAAATTGGCA	Sus/Res upstream *super-kdr* primer
FG-154	ACCCATTGTCCGGCCCA	Sus downstream *super-kdr* diagnostic
FG-155	ACCCATTGTCCGGCCCG	Res downstream *super-kdr* diagnostic
FG-236	TTGTTGTCATGCTGCCTCC	Sus/Res upstream αE7 primer
FG-238	CGCCACAAATGAAACC	Sus downstream αE7 primer
FG-239	GCCACAAATGAAACG	Res downstream αE7 primer
FG-240	GCCACAAATGAAACA	Res downstream αE7 primer
FG-241	GCCACAAATGAAACT	Res downstream αE7 primer
FG-243	GGCATGGCTTTCCGTGTCC	GAPDH PCR-positive control primer
FG-234	CTTCTTCATCGGTGTAGC	GAPDH PCR-positive control primer
FG-129	AGGACAAATTCAAAGATCATG	*kdr* upstream primer for nested PCR protocol
FG-158	AGATTTAAGGCTCCACAAC	Upstream primer for αE7 fragment cloning
FG-327	ATAATCAGCGCCATAATAG	Downstream primer for αE7 fragment cloning

Res, resistant; Sus, susceptible.

and *superkdr* sodium channel mutations and a resistance-associated Gly^{137}→Asp mutation in the carboxylesterase *HiαE7.* Individual flies were genotyped using PCR to determine the presence or absence of these mutations (Jamroz et al. 1998, Guerrero 2000). The assay has been described in detail by Li et al. (2003). Briefly, genomic DNA was purified from individual flies by an adaptation of a method used for DNA purification from *Drosophila melanogaster* (Czank 1996). A disposable pellet pestle for 1.5-ml centrifuge tubes (Kontes, Vineland, NJ) was used to crush and grind individual flies. Twenty five microliters of sample buffer (100 mM Tris, pH 8.3, and 500 mM KCl) was added to the tube and grinding continued for ≈20 s. The tube contents were briefly microcentrifuged and placed in a boiling water bath for 3 min. After cooling, 2.5 μl of a 1:10 dilution in water (100–150 ng of DNA) was used for PCR with a reaction mixture containing primers as described below, 10 mM tris(hydroxymethyl)aminomethane hydrochloride, pH 8.3, 50 mM KCl, 0.05 mM each dNTP, 2 mM $MgCl_2$, and 0.1 μl of a 1:1 (vol:vol) mix of *AmpliTaq* DNA polymerase (5 U/μl stock; Applied Biosystems, Foster City, CA) and TaqStart Antibody (1.1 μg/μl stock; Clontech, Palo Alto, CA). Primers were used at the following concentrations: FG-130, -134, -138, -234, -235, -154, -155, and -243, 1 μM; and FG-236, -238, -239, -240, and -241, 0.5 μM (Table 2). Each complete PCR pyrethroid resistance genotyping assay requires two amplification reactions. To assay for the presence of susceptible alleles, only primers FG-130, -138, -154, -235, -236, and -238 were included in the reaction mix. To assay for resistant mutated alleles, only primers FG-134, -138, -155, -235, -236, -239, -240, and -241 were included in the reaction mix. Primers FG-234 and -243 are included in both assays, because they serve as PCR-positive controls and amplify a portion of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene coding region. Amplification was carried out using a DNA Engine (MJ Research, Watertown, MA) programmed for 96°C for 2 min followed by 35 cycles, each consisting of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. The program also included a final extension step at 72°C for 7 min. Reaction products were fractionated on agarose Tris borate-EDTA gels, and DNA was visualized by staining with GelStar DNA Staining Dye (FMC Bioproducts, Rockland, ME) followed by UV illumination. The sizes of the diagnostic PCR products for the *kdr*, *super-kdr*, and *αE7* alleles were 285, 72, and ≈250 bp, respectively. The size of the GAPDH-positive control PCR product was 154 bp.

A nested PCR strategy was used to determine the *kdr* genotype for templates that amplified poorly in the multiplex protocol. A primary PCR was performed using 0.5 μl of undiluted genomic DNA solution from a single fly: primers FG-129, -138, -234, and 243, each at 1 μM, 10 mM tris(hydroxymethyl)aminomethane hydrochloride, pH 8.3, 50 mM KCl, 0.05 mM each dNTP, 2 mM $MgCl_2$, and 0.1 μl of a 1:1 (vol:vol) mix of *AmpliTaq* DNA Polymerase and TaqStart Antibody. Amplification was carried out using 96°C for 2 min followed by 43 cycles, each consisting of denaturation at 94°C for 1 min, annealing at 62°C for 1 min, extension at 72°C for 1 min, and a final extension step at 72°C for 7 min. A secondary PCR was performed using 1 μl of the primary PCR product as template, 10 mM tris(hydroxymethyl)aminomethane hydrochloride, pH 8.3, 50 mM KCl, 0.05 mM each dNTP, 2 mM $MgCl_2$, and 0.1 μl of a 1:1 (vol:vol) mix of *AmpliTaq* DNA Polymerase and TaqStart Antibody. To detect the presence of susceptible *kdr* alleles, primers FG-130 and -138 were included at 1 μM. To detect the presence of resistant *kdr* alleles, primers FG-134 and -138 were included at 1 μM. Amplification was carried out using 96°C for 2 min followed by 43 cycles, each consisting of denaturation at 94°C for 1 min, annealing at 66°C for 1 min, extension at 72°C for 1 min, and a final extension step at 72°C for 7 min.

To verify the αE7 genotype, a fragment encoding amino acids 72–152 was amplified using 0.5 μl of genomic DNA from an individual fly diluted 1:10 in water, primers FG-158 and -327, each at 1 μM, 10 mM tris(hydroxymethyl)aminomethane hydrochloride, pH 8.3, 50 mM KCl, 0.05 mM each dNTP, 2 mM $MgCl_2$,

Table 3. Results from *αE7*, *superkdr*, and *kdr* PCR genotyping of Brazilian horn fly populations

Location	αE7		Sodium channel[a]						
	n[b]	Genotype	n	*superkdr* genotype			*kdr* genotype		
				SS	SR	RR	SS	SR	RR
Ciaco		—	9	9	0	0	9	0	0
Nhumirim (2000)		—	10	10	0	0	10	0	0
Monjolo	10	All wild type	10	10	0	0	10	0	0
Cristo			50	50	0	0	50	0	0
Santa Cruz			50	50	0	0	50	0	0
Taboco			31	31	0	0	30	1	0
Estância Sta Izabel			37	37	0	0	36	1	0
Nhumirim (2004)	9	All wild type	17	17	0	0	17	0	0
Iguaçu	6	All wild type	25	25	0	0	25	0	0
Bosque Belo	23	All wild type	25	25	0	0	24	1	0
Santa Izabel	12	All wild type	25	25	0	0	25	0	0
Estrela do Oeste			50				25	23	2
Barra Bonita			48				45	3	0

[a] S denotes a pyrethroid susceptible-associated allele, and R denotes a pyrethroid resistance-associated allele. Two alleles per individual are assumed with SS denoting a homozygous susceptible genotype, RR denoting homozygous resistant genotype, and SR denoting the heterozygous state.

[b] Denotes number of individual flies assayed.

and 0.1 μl of a 1:1 (vol:vol) mix of *AmpliTaq* DNA Polymerase and TaqStart Antibody. Amplification was carried out using 96°C for 2 min followed by 35 cycles, each consisting of denaturation at 94°C for 1 min, annealing at 64°C for 1 min, extension at 72°C for 1 min, and a final extension step at 72°C for 7 min. The 243-bp fragment was gel purified using the QIAquick gel extraction kit (QIAGEN, Valencia, CA), concentrated with Pellet Paint Co-Precipitant (Novagen, Madison, WI), cloned with the PCR-Script Amp cloning kit (Stratagene, La Jolla, CA), and sequenced.

Results

Bioassays from ranches sampled in 2000 showed low levels of permethrin resistance (Table 1). Ciaco, Nhumirim, and Monjolo had statistically significant differences in LC_{50} compared with the Kerrville susceptible population and had RFs of <2.0, whereas Cristo, sampled in 2001, was susceptible to permethrin (RF = 0.8). All populations assayed in 2002 or 2004 displayed statistically significant differences in LC_{50} compared with the Kerrville susceptible population and had elevated RFs against cypermethrin. TPP synergist studies conducted in 2004 on Nhumirim, Iguaçu, Santa Izabel, Barra Bonita, and Bosque Belo showed no statistically significant TPP synergism of cypermethrin. However, when TPP and cypermethrin were tested against the horn fly population at Estrela do Oeste, a 45% decrease in cypermethrin RF was found.

No evidence of the *superkdr* sodium channel gene mutation was detected in these populations, but the *kdr* mutation was detected in five of the populations (Table 3). In four of these populations, the frequency of individual flies with a *kdr* allele was below 7%. However, the Estrela do Oeste population was made up of 46% heterozygote and 4% homozygous resistant individuals at the *kdr* locus. There was no evidence for the resistance-associated $Gly^{137} \rightarrow Asp$ mutation in *HiαE7* in any population. All PCR assays showed the wild-type susceptible *HiαE7* genotype. To verify the PCR results, seven individuals were selected for direct DNA sequencing of the gene coding region containing the codon 137. Two individual flies were selected from each of the cypermethrin-resistant (Table 1) Iguaçu, Santa Izabel, and Bosque Belo populations and one fly from the cypermethrin-resistant Nhumirim population. One of the Bosque Belo flies had the SR heterozygous *kdr* and SS susceptible *superkdr* genotype, and all other flies had the SS susceptible *kdr* and *superkdr* genotypes in the PCR assay (Table 3). The wild-type Gly-encoding sequence (GGT) was found at codon 137 in all cases.

Information gathered from the questionnaire showed that use of pyrethroids for controlling horn flies was common throughout the state. Insecticides were applied primarily with backpack manual sprayers at most ranches, and application rates per animal were considerably lower than that recommended on the label. All cattle ranches where bioassays were carried out had a history of primarily using cypermethrin and deltamethrin, with the exception of Nhumirim. At Nhumirim, horn flies are not controlled for research purposes but neighboring ranches used insecticides for fly control.

Discussion

Most published reports of pyrethroid resistance in the horn fly note significant contributions from reduced target site sensitivity, presumably resulting from the *kdr* mutation. Recent surveys which incorporated the *kdr* PCR assay have shown the *kdr* mechanism to be prevalent in several parts of the United States (Jamroz et al. 1998, Foil et al. 2005), Mexico (Li et al. 2003), and Argentina (Guglielmone et al. 2002). However, in our study the high levels of cypermethrin resistance, without appreciable levels of *kdr* mutations, were surprising. It is possible that the lack of widespread use of ear tags, as occurred in the United

States, as well as the frequent use of suboptimal doses and concentrations of insecticide may have favored selection of metabolic mechanisms and resulted in a slower spread of target site pyrethroid resistance through the region of Brazil sampled during our study. Compared with the situation in the United States and Mexico, the horn fly is a recently introduced species to Brazil, and insecticide resistance is a recent phenomenon. In the absence of significant levels of *kdr* allele-mediated target site resistance, metabolic resistance mechanisms may be one of the first lines of defense available to the fly for responding to insecticide challenge, making use of enzymes (e.g., mixed function oxidases, esterases and glutathione *S*-transferases) already present within all individual flies of a population. Changes in the sodium channel gene sequence, responsible for target site resistance, are a different type of defensive response, less immediate, and show up at the population level in later generations as individuals with favorable gene mutations in the insecticide target site spread the new allele under the selection pressure of the pyrethroid. The spread of *kdr* alleles through the Brazilian horn fly population offers an opportunity to view the introduction of a new allele into a susceptible natural population and to monitor its spread with a simple genetic assay.

Moreover, enhanced toxicity of cypermethrin in the Estrela do Oeste bioassays combined with TPP strongly suggests the participation of esterases as a component in the mechanism of pyrethroid resistance in this population. Thus, the high level of resistance found in this population seems to have resulted from at least two mechanisms: kdr and increased esterase activity. A significant role for both mechanisms has been previously found in horn fly populations (Xu and Bull 1995, Jamroz et al. 1998). We should point out that in four of the six synergist bioassays, the RF was higher in the presence of TPP than in its absence (Table 1). The Kerrville reference strain has been found to have a general esterase-based component in its response to pesticide, which is synergized by TPP (F.G., unpublished data). Thus, a lower value in the RF equation denominator will contribute to a higher RF value. Regardless, only the Estrela do Oeste population shows a significant difference in LC_{50} value between the synergized and unsynergized bioassays. The lack of the $Gly^{137} \rightarrow Asp$ mutations in the *Hi*α*E7* gene was not surprising, because this mutation has not been found in surveys of insecticide susceptible and resistant horn flies from the United States and Mexico (F.G., unpublished data). Although this mutation was first identified and shown to directly lead to diazinon resistance in *Lucilia cuprina* (Weidemann) (Newcomb et al. 1997) and *Musca domestica* L. (Claudianos et al. 1999), findings by Sabourault et al. (2001) led this group to propose a model whereby the nonmutated αE7 regulates the titer of a *trans*-acting factor that negatively controls the complex expression of a number of genes, including members of the cytochrome P450 family of metabolic oxidases and the glutathione *S*-transferases. Both of these gene families have members that have been implicated in metabolic mechanisms of insecticide resistance, and it was conceivable they could play a role in the pyrethroid resistance mechanism of the horn flies from Mato Grosso do Sul. Thus, when the *kdr* target site mechanism did not seem to play a major role, if any, in these populations, we were very interested in verifying the *Hi*α*E7* genotype.

It is also possible that an alternative target site insensitivity mechanism is present in these flies that is mediated by a sodium channel mutation other than the Leu→Phe mutation assayed for by this PCR test. Some insects have been shown to possess sodium channel mutations at sites other than the classic Leu→Phe position in domain II (Soderlund and Knipple 2003). We feel this possibility is unlikely, because the Leu→Phe mutation is common in horn fly populations in the United States, Mexico, and Argentina and was also found in Central Brazil, particularly the Estrela do Oeste population. Although frequency of *kdr* flies does not completely explain resistance levels found in bioassays, it likely plays a role in horn fly pyrethroid resistance in most populations. However, in our study, both mechanisms (*kdr* and esterases) failed to explain the resistance levels detected in bioassays, suggesting the existence of another mechanism having a major role in pyrethroid resistance in horn fly populations in the state of Mato Grosso do Sul. Studies using insecticide synergized with PBO are being conducted and are expected to bring useful information to elucidate possible oxidative metabolic mechanisms behind pyrethroid resistance in the state.

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